II. REMARKS

Formal Matters

Claims 1, 3, 5, 7, and 9-22 are pending after entry of the amendments set forth herein.

Claims 1-4, 14, and 15 were examined. Claims 1-4, 14 and 15 were rejected. Claims 5-13 and 16-19 were withdrawn from consideration.

Claims 1, 3, 5, 7, 14, and 15 are amended. The amendments to the claims were made solely in the interest of expediting prosecution, and are not to be construed as an acquiescence to any objection or rejection of any claim. Support for the amendments to claims 1, 3, 5, 7, 14, and 15 is found in the claims as originally filed (e.g., originally filed claims 2 and 4), and throughout the specification, in particular at the following exemplary locations: Example 1; paragraph 0082; and paragraph 00194. Accordingly, no new matter is added by these amendments.

Claims 2, 4, 6, and 8 are canceled without prejudice to renewal, without intent to acquiesce to any rejection, and without intent to surrender any subject matter encompassed by the canceled claims. Applicants expressly reserve the right to pursue any canceled subject matter in one or more continuation and/or divisional applications.

Claims 20-22 are added. Support for new claims 20-22 is found in the claims as originally filed, and throughout the specification, including the following exemplary location: paragraph 0082. Accordingly, no new matter is added by new claims 20-22.

Applicants respectfully request reconsideration of the application in view of the remarks made herein.

Examiner Interview

The undersigned Applicants' representative thanks Examiner Ton for the courtesy of a telephonic interview, which took place on January 21, 2004, and which was attended by Applicants' representative Paula A. Borden and Examiner Ton. During the interview, possible amendments to claims 1 and 5 were discussed, and the rejection under 35 U.S.C.§103 was discussed. It is the understanding of the undersigned Applicants' representative that Examiner Ton agreed to examine claims 5-8, which were previously withdrawn from consideration.

Claim objection

Claim 15 was objected to as depending from itself.

Claim 15 is amended to depend from claim 14, thereby adequately addressing this objection.

Rejection under 35 U.S.C.§112, first paragraph

Claims 1-4, 14, and 15 were rejected under 35 U.S.C.§112, first paragraph, as allegedly lacking enablement.

The Office Action stated that the claims are enabled for a transgenic mouse whose genome comprises an endogenous mouse apolipoprotein E4 (apoE4) allele operably linked to endogenous regulatory sequences, wherein the endogenous apoE4 allele has been modified by the substitution of an arginine residue with a threonine residue at the position equivalent to amino acid 61 of human apoE4, and wherein the expression of the modified endogenous mouse apoE4 results in the preferential binding of lower density lipoproteins. The Office Action stated that the specification does not enable any person skilled in the art to make and/or use the invention commensurate in scope with the claims. Applicants respectfully traverse the rejection.

The Office Action stated that the stated of the art of ES cells from species other than mice is unpredictable and undeveloped, and stated that it would have required undue experimentation for one of skill in the art to make and/or use the claimed non-human animals and methods of using the same. Applicants respectfully disagree. The art of manipulating the genome of a variety of non-human mammals is well developed. As of the priority date of the instant application, those skilled in the art knew how to make gene-targeted non-human mammals[1].

As further evidence for the fact that methods of altering the genome of non-human animals of various species is not "unpredictable and undeveloped," Applicants note that several U.S. patents have issued, which claim non-human mammals whose genome has been altered and/or methods for making

[1]As of the priority date, numerous publications were available, teaching how to make transgenic animals, including, e.g., Transgenesis Techniques: Principles and Protocols D. Murphy and D.A. Carter, ed. (June 1993) Humana Press; Transgenic Animal Technology: A Laboratory Handbook C.A. Pinkert, ed. (Jan. 1994) Academic Press; Transgenic Animals F. Grosveld and G Kollias, eds. (July 1992) Academic Press; and Embryonal Stem Cells: Introducing Planned Changes into the Animal Germline M.L. Hooper (Jan. 1993) Gordon & Breach Science Pub.

such animals. See, e.g., U.S. Patent Nos. 6,518,482, 6,339,183, 6,268,545, 6,255,554, 6,222,094, 6,204,431, and 5,850,000. Issued U.S. patents are presumed valid, and as such are evidence that it is the Office's position that methods of manipulating the genome of a variety of non-human mammals are not unpredictable.

Nevertheless, and solely in the interest of expediting prosecution, claim 1 is amended to recite "a gene-targeted mouse."

Applicants submit that the rejection of claims 1-4, 14, and 15 under 35 U.S.C.§112, first paragraph, has been adequately addressed in view of the remarks set forth above. The Examiner is thus respectfully requested to withdraw the rejection.

Rejections under 35 U.S.C.§112, second paragraph

Claims 1, 2, 14, and 15 were rejected under 35 U.S.C.§112, second paragraph, as allegedly indefinite.

Claim 1

The Office Action stated that claim 1 is unclear, and stated that the claim fails to provide a clear definition of what characteristics of human apoE4 the modified allele exhibits. Applicants respectfully traverse the rejection.

Claim 1 recites that the modified apoE exhibits domain interaction characteristic of human apoE4. The specification provides ample description of apoE4 domain interaction, and how to determine whether an apoE protein exhibits domain interaction. For example, the specification discusses domain interaction as involving formation of a salt bridge between an Arg residue at about amino acid 61 and a Glu residue at about amino acid 255. Specification, paragraph 0067. The specification also describes an emulsion binding assay to assess domain interaction. Specification, paragraph 00133. Accordingly, claim 1 is clear and need not be amended.

Nevertheless, and solely in the interest of expediting prosecution, claim 1 is amended to recite "wherein the modified apoE polypeptide exhibits preferential binding to lower density lipoproteins."

Claim 2

The Office Action stated that claim 2 is unclear, and stated that a position *equivalent* to amino acid 61 fails to clearly define the modification that occurs on the endogenous apoE4 allele. Applicants respectfully traverse the rejection.

Claim 2 is canceled without prejudice to renewal, thereby rendering this rejection moot.

Claim 14

The Office Action stated that claim 14 is incomplete, and stated that it is unclear how the determination of an effect of a test agent on a phenomenon associated with AD relates to the preamble.

Without conceding as to the correctness of this rejection, claim 14 is amended to recite "determining the effect of the test agent on reducing a phenomenon associated with AD."

Claim 15

The Office Action stated that claim 15 depends from itself.

Claim 15 is amended to depend from claim 14, thereby adequately addressing this rejection.

Conclusion as to the rejections under 35 U.S.C.§112, second paragraph

Applicants submit that the rejections under 35 U.S.C. §112, second paragraph have been adequately addressed in view of the remarks set forth above. The Examiner is thus respectfully requested to withdraw the rejection.

Rejection under 35 U.S.C.§103

Claims 1-4, 14, and 15 were rejected under 35 U.S.C.§103 as allegedly unpatentable over Capecchi ((1994) *Sci. American* 270:34-41; "Capecchi") when taken with Dong et al. ((1994) *J. Biol. Chem.* 269:22358-22365; "Dong") and Weisgraber et al. ((1994) *Adv. Protein Chem.* 45:249-302; "Weisgraber").

The Office Action stated: 1) Capecchi teaches knockout technology applied to mice, and discloses the applicability of gene targeting to many other genes; 2) Capecchi differs from the claimed invention in that the targeting construct does not contain flanking nucleotide sequences which homologous recombine with the endogenous mouse apolipoprotein E4 to produce a Thr → Arg substitution at the position equivalent to amino acid position 61 of human apoE4; 3) Dong teaches that human apoE has been implicated in the development of Alzheimer's Disease (AD), and teaches that Arg-61 is critical in determining apoE lipoprotein preference; and 4) Weisgraber teaches the primary structures of apoE from 10 species, including mouse. The Office Action concluded that it would have been obvious to utilize the transgenic technology as taught by Capecchi, with a targeting vector comprising a modified apoE4 allele with a Thr → Arg substitution at a position equivalent to amino acid 61 of human apoE4 to produce transgenic mice, with a reasonable expectation of success. Applicants respectfully traverse the rejection.

Capecchi teaches nothing about apoE4 domain interaction. Capecchi teaches nothing about which residues in apoE4 protein would be involved in domain interaction. Capecchi teaches nothing about modifying the genome of a non-human animal such that a modified apoE protein is produced that exhibits domain interaction characteristic of human apoE4. Accordingly, Capecchi cannot render claims 1-4, 14, and 15 obvious.

Weisgraber does not cure the deficiency of Capecchi. Weisgraber provides the amino acid sequences of apoE from various species.

Dong also does not cure the deficiency of Capecchi. Dong discusses the importance of Arg-61 in binding VLDL, and notes that humans are unique in having Arg at position 61 in apoE. Nowhere does Dong suggest creating a gene-targeted mouse having an endogenous apoE allele modified such that the codon for Thr-61 is substituted with a codon for Arg. Accordingly, Capecchi, alone or in combination with Weisgraber and Dong, cannot render claims 1-4, 14, and 15 obvious.

The Office Action states that there was a reasonable expectation of success that one could generate a gene-targeted mouse that has a modified apoE allele encoding apoE with a Thr → Arg substitution, with a reasonable expectation of success. Applicants agree that such animals could be generated with a reasonable expectation of success. However, the cited art provides no reasonable

expectation of success that such animals would produce a modified apoE that exhibits domain interaction characteristic of human apoE4. As shown in Figure 1 of Weisgraber, the amino acid sequence of mouse apoE differs from that of human apoE4 at many amino acid positions. In fact, over a stretch of 299 amino acids, the are 89 amino acid sequence differences between mouse and human apoE. There was no reasonable expectation in the cited art that, merely by altering the sequence of mouse apoE by *one amino acid*, that the modified apoE would exhibit domain interaction characteristic of human apoE4 (e.g., preferential binding to lower density lipoprotein) *in vivo*.

The Office Action stated that Dong provides motivation to make the modification, citing a statement in Dong to the effect that the fact that no animal model appears to develop the complete pathology of Alzheimer's disease may result from the uniqueness of human apoE, specifically the presence of arginine at position 61. In citing this statement in Dong, the Office Action appears to be applying an "obvious to try" rationale in support of the obviousness rejection. However, "obvious to try" is not the standard under 35 U.S.C. §103.

In summary, the primary reference, Capecchi, does not teach or suggest anything about apoE or domain interactions. Neither Weisgraber nor Dong cure the deficiencies of Capecchi. Furthermore, there was no reasonable expectation of success that of generating a gene-targeted non-human animal that produces a modified apoE that exhibits the domain interaction characteristic of human apoE4. Accordingly, Capecchi, alone or in combination with Weisgraber and Dong, cannot render claims 1-4, 14, and 15 obvious.

Nevertheless, and solely in the interest of expediting prosecution, claim 1 is amended to recite "wherein the modified apoE polypeptide exhibits preferential binding to lower density lipoprotein." As discussed during the Examiner interview, the phenotype of exhibiting preferential binding to lower density lipoprotein is not predictable based on the cited art. It was not predictable that, merely by altering the sequence of mouse apoE by *one amino acid*, the modified apoE would exhibit preferential binding to lower density lipoprotein.

Applicants submit that the rejection of claims 1-4, 14, and 15 under 35 U.S.C. §103 has been adequately addressed in view of the remarks set forth above. The Examiner is thus respectfully requested to withdraw the rejection.

III. CONCLUSION

Applicants submit that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number UCAL222.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: Jan. 23, 2004

By: _____
Paula A. Borden
Registration No. 42,344

BOZICEVIC, FIELD & FRANCIS LLP
200 Middlefield Road, Suite 200
Menlo Park, CA 94025
Telephone: (650) 327-3400
Facsimile: (650) 327-3231

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